PHOTO-MICROGRAPHY

By Captain A. D. G. SHELLEY, R.E.

A Communication to the Amateur Photographic Society of Madras.

1. The representation of microscopic objects was one of the earliest applications of photography, and even as far back as 1802 fugitive photo-micrographic impressions on paper and leather were obtained by Sir Humphry Davy and Wedgewood. At first great things were expected from the new power thus brought into play, but as results fell short of anticipations the process came to be looked on with a certain amount of disfavour. Now, however, that a juster appreciation of the capabilities of photography prevails, this manner of delineating microscopic objects is much used, and many beautiful photo-micrographs are existant, shewing a delicacy and fullness of detail which could not have been obtained by any other process. In a short paper like the present it is only possible to deal with photo-micrography in a very sketchy way, but it is hoped that even the meagre information given in this paper may induce some Members of the Society to take up a very fascinating branch of photography.

2. Some slight knowledge of the microscope is necessary before any attempt can be made to use the instrument in conjunction with photography, and of course before any really good work can be done a considerable amount of facility in using the

instrument and in the management of light must be attained. However as very little practice, and a not expensive outfit will enable many interesting photographs to be taken, it is hoped that beginners may not be scared by the imposing outfit recommended in the catalogues of many dealers in scientific instruments.

3. The picture of a microscopic object should shew all the details with as perfect distinctness as possible, but where the object to be represented has any appreciable thickness, the whole of the details are only visible through the microscope by successively changing the focus of the instrument to suit the principal planes of the subject. A microscopic illustration is consequently a diagram shewing as if they were simultaneously visible, details which can only be seen at different focal distances. These requirements impose a limit to the application of photography, for if we attempt to change the focus of the microscope during exposure a confused negative will be the only result. It is therefore obvious that the flatter an object is the more suitable is it for photographic reproduction under the microscope, and that it is hopeless to try and take in one operation a subject having much relief. The greater the magnification employed, the more limited is the depth of focus, that is the thinner is the portion of the object clearly visible at one time. Hence, irrespective of the difficulties in the manipulation of high powers, a flat object is the easiest to photograph, where the structure can be properly shewn under low magnification. Good photographs can however be obtained under very great amplification with skilled manipulation and suitable lenses, for the reason that high power subjects are very thin, and that one position of focus embraces all the planes necessary for serviceable representation. The most satisfactory field for photo-micrography lies at the extremes of amplification from, say, magnifications of 20 to 70, and from 500 to 1,500 diameters. Subjects

requiring objectives of $\frac{1}{4}''$ to $\frac{1}{6}''$ focus for their examination will probably be found the most difficult to photograph.

4. Although medium power objects cannot be photographed to advantage in one operation, it is of course possible to take a series of negatives of the most important planes and combine the results into one picture. In delicate subjects this is not very easy, but it is worth trying especially if the operator has facility in drawing. Another method adopted with success consists in painting out on the negative the indistinct portions, and taking an under-exposed print on smooth bromide paper. This positive is used as the basis of a diagram which can be filled in by hand, and which when worked up in Indian ink and pencil presents the appearance of an elaborately finished drawing. A treatment of the difficulty, which has been recommended by some authorities, is to take in the first instance a photograph under a low degree of magnification and afterwards enlarge it by any ordinary photographic copying process. This method has the advantage of shortening the time of exposure, but necessitates the focus of the original negative being extremely sharp.

5. Photography as applied to microscopy is further complicated by the differences in transparency, and the great contrasts in color which are frequently present in the same object. In ordinary photography the first difficulty is not met with as objects are illuminated by reflected light, and variations in opacity do not influence the quality of the negative. In the microscope, however, the majority of preparations can only be viewed by transmitted light, that is light which passes through the object, so that the resulting negative is very largely affected by the color contrasts and the variations in transparency of the subject to be photographed. The yellow color of many anatomical specimens so interferes with the transmission of light as to almost preclude the possibility of making a photograph. Again,

the thinness with which modern sections are usually cut renders them too actinically transparent to be photographed with low powers, but at the expense of prolonging the exposure from three to four times, this may be overcome by the interposition of a screen of yellowish green glass. Where great differences in color are present in the same preparation, or where non-actinic colors, such as deep brown, prevail, orthochromatic plates offer decided advantages. Many dark colored objects, such as insects, may be bleached by prolonged maceration in turpentine, while sections of dark woods may be treated in nitric acid.

6. The only absolutely essential apparatus required for photo-micrography are a few good object glasses, with some type of stage and illuminating arrangement, and some form of camera or its equivalent, whereby the picture formed by the objective may be received on a sensitized plate. A very large number of arrangements have been devised to meet these requirements, but it is impossible in this paper to more than discuss them in a general manner. It will be convenient to consider photo-micrographic apparatus as divided into four classes as under:—

(*a*) The attachment of a special camera to an ordinary compound microscope.

(*b*) The combination of an ordinary camera with a compound microscope.

(*c*) The substitution of a dark room for the camera and the use either of an ordinary compound microscope, or a system consisting of stage, objective, and mirror.

(*d*) A combination camera and microscope forming one piece of apparatus.

7. A very simple method of effecting the first arrangement (*a*) is by attaching a pyramidal light tight box to the microscope, the small end of the box fitting closely to the eye-piece, and

the large end being adapted in the usual way to take a focussing screen and a dark slide. The focussing is effected by the adjusting screws of the microscope, which can be used either with the body horizontal or vertical.

8. The second arrangement (*b*) is still easier for all who possess an ordinary photographic camera. It merely consists in the insertion of the eye-piece end of a compound microscope placed with the body horizontal into the lens aperture of the camera. The space between the microscope and the wood work of the front must be carefully closed to exclude light. When working with lamp-light any black cloth packed well into the space will answer all purposes. The eye-piece of the microscope may or may not be removed, it is simpler not to do so, as its presence permits of a short camera being used and the focussing screws of the microscope remain within reach of the hand. Most of the well known Microscopists recommend the removal of the eye-piece, as its use involves a loss of light, but in this case some mechanical arrangements must be devised to actuate the focussing screws which will not be in reach. Doctor Woodward in place of the eye-piece employs a special combination which he calls an achromatic concave. Doctor R. Neuhauss has found that the ordinary eye-piece can be used instead of a projection ocular for throwing the picture on the focussing screen. The lenses of the eye-piece are separated a little distance and an additional diaphragm is fitted on. The arrangement is simple, a paper case or tube, 2½ cm., long, is fitted on to the brass tube, the internal diaphragm remains in its original position, while the new one is fitted over the eye-piece by means of a short movable tube. The nearer the objects to be photographed are to the focussing screen, the further must the lenses of the eye-piece be removed from one another.

9. The third arrangement (*c*) may be exemplified by the plan adopted by Mr. Wenham who dispenses with a camera and

uses instead a dark-room. This room he closes with a shutter having a circular aperture 3 inches in diameter : upon the outside of the aperture is placed a reflector of some type, which can be actuated from inside the room. The microscope body is arranged horizontally on a table or bench, so that its axis if prolonged would pass through the centre of the hole in the shutter. The object to be photographed is suitably placed on the stage of the microscope and near to the inside of the aperture, the light around the stage being cut off by a black cloth. A vertical stand, grooved to carry a sensitized plate or a white card, completes the arrangement. The enlarged view of the object to be photographed is first projected on to the white card and focussed, the light is then cut off and the sensitized plate is inserted in the grooves which held the card. The plate is then exposed by re-admitting the light for the necessary time.

10. The fourth arrangement (*d*) need only be briefly referred to. A stage with mirror and objective is attached to the front of a special camera provided with necessary fittings for adjustment.

11. The next point to be considered is illumination. The sun, the electric, oxy-hydrogen, magnesium or zircon light, and the ordinary parafin or petroleum flame are the usual sources of illumination. Of these the majority of operators prefer sunlight, but in using it with any form of condenser care must be taken to guard against the intense heating effects of the rays which are liable to injure the object, and even in the case of high power objectives to uncement the lenses. In the case of subjects requiring long exposure it will be necessary to supplement the apparatus previously enumerated by a heliostat in order that the solar beam may be reflected in a constant direction, without a heliostat rapidity of impression is absolutely necessary for the most perfect definition. Various devices have been resorted

to for overcoming the heating effects of the condensed solar rays, the most common plan being to reflect the light through a cell filled with a solution of sulphate of copper, the blue color of which filters off the heat rays while allowing the actinic components of the solar beam to pass through. Another plan is to break up the light by means of a large prism of wide dispersion and then intercept the rays of the red end of the spectrum. In this way the defects of chromatic aberration may be avoided, and a more perfect definition obtained. A very ingenious method proposed by Mr. Reade for using his hemispherical condenser with a solar condenser is given in "How to work with the microscope," by Dr. Beale. The rays furnishing light and those giving off heat having different degrees of refrangibility, we have here the cone of light rays formed within the cone of heat rays so that when these rays cross the axis, their respective positions are reversed. The hemispherical lens is so arranged that it is separated from the principal focus of the heat rays by its own focal length, when the principal focus for light will be found at a greater distance than that for heat; the heat rays will thus be rendered parallel while the light rays will converge to a second focus furnishing an intense light unaccompanied by heat. The same object is effected by Dr. Woodward by placing an achromatic lens at such a distance from the achromatic condenser of the microscope that the solar rays are brought to a focus and begin again to diverge before they reach the lowest glass of the condenser. This method is so successful that enough light can be obtained to give excellent definition on a card board screen under a magnification of 5000 diameters.

12. When using sunlight it will sometimes be found that diatoms and soft tissues when illuminated with parallel rays will produce interference lines. A ground glass screen, preferably greased, interposed between the mirror and the condenser

will remedy this inconvenience. The electric light by exaggerating the effects of light and shade is well suited for delicate objects possessed of little contrast. In using this illumination the pencil of light should be first rendered parallel by means of the condenser usually supplied with electric lamps for this purpose, and then condensed as with solar light. The electric light is said by some authorities to be cumbrous, unsteady, expensive and troublesome, but these remarks would appear to be directed against the arc light as the incandescent lamp seems free from these objections. The magnesium and oxy-hydrogen lights are of special service in photographing soft tissues, and no interference phenomena presenting the greased glass screen can be dispensed with. The light is concentrated on to the lower lens of the achromatic condenser, and the ammonia-sulphate cell should invariably be used. The fumes of magnesia, which give trouble by the deposits on surrounding objects, may be collected on a muslin chimney made by covering a spiral wire column 5 feet long, the cloth being folded in similar fashion to the bellows of a camera. The zirconia light is produced by placing in the oxy-hydrogen flame a mass made by strongly heating in an iron mould, a paste composed of zirconia mixed with a solution of boracic acid. This illumination is even more brilliantly luminous than lime light. Zirconium is very resistant and gives a regular steady flame. It will be found however that petroleum light is sufficient for almost all purposes and any good lamp may be used, but the ordinary microscopic lamp which can be raised or lowered bodily will be found most convenient. A very intense illumination may be obtained from paraffin oil by using it in a triple wick lamp with a condenser to parallelize the rays. This pencil will of course have to be rendered convergent before it enters the optical portion of the microscope. With all lighting the achromatic condenser must be carefully centred as otherwise unequal illumination will result.

After centring, the condenser must be moved back until the field is uniformly lighted.

13. Certain objects, such as diatoms of close striation, require the use of very oblique light which can be obtained in the following way, given in Davis' "Practical Microscopy." "A parallel pencil of solar rays from the heliostat and plane mirror is intercepted by a blue cell and diaphragm which only allows a circular pencil of half an inch diameter to pass. The light enters parallel to the optic axis of the microscope placed in the usual position for photography, but at a lateral distance to the right or left of 3 inches. If the light is intercepted by a large achromatic prism of a focal length of about 3 inches, the desired obliquity can be obtained without difficulty. The best result is obtained when the rays are concentrated to a focus upon the object, and it is indispensable that the stage of the microscope should be as thin as possible. The illumination thus obtained is in general sufficient to produce negatives by the wet process up to 2500 diameters with three minute's exposure."

14. The magnified image of the object to be photographed can be focussed by projecting it on to a white screen, or on to a ground or plane glass plate, or by examining it with the ordinary microscopic eye-piece. The ground glass usually supplied with the camera is too coarse for fine focussing and should be re-ground with the finest emery and water, or a focussing screen may be made by coating an ordinary glass plate with the following varnish recommended by Davis :—

Gum mastic	...	...	40 grains
Gum sandarac	...	...	160 grains.
Ether ...	...	...	4 oz.
Benzol ...	...	...	1½ oz.

This varnish is not easy to apply, as even the heat of the fingers is sufficient to make the coating immediately over them dry with a smooth instead of a matt surface.

If a piece of plate glass be used the image is viewed by a focussing glass or eye-piece held against the plate glass, the focus of the lens corresponding exactly with the anterior surface of the plate. The simplest and, I believe, the best method of focussing is to use the ordinary low power eye-piece inserted into a series of apertures in a thin wooden board substituted for the usual ground glass, care being taken that the diaphragm of the eye-piece is in the exact position that will be occupied by the film side of the sensitized plate.

15. In connection with focussing it is to be noted that objectives being over-corrected, it frequently happens with low and medium powers that the actinic and visual foci are not coincident. The result of this is, that a blurred and indistinct negative may be obtained although the image viewed by the eye when focussing shewed perfect definition. This drawback can be remedied by having the objectives specially corrected for photography, or by withdrawing the object glass after focussing a certain amount determined by experiment. The number of turns of the fine adjustment screw necessary to get the chemical focus is easily obtained by developing a negative taken at the best visual focus, and then withdrawing the objective till the image appears to the eye as indistinct as it is on the negative. I append the amount of correction required by various objectives which have been extracted from Beale's and Davis' works.

Beck	$1\frac{1}{2}$	inch	$\frac{1}{50}''$	Browning	4	inch	$\frac{1}{100}$
„	$\frac{2}{3}$	inch	$\frac{1}{200}''$	„	1	inch	$\frac{1}{400}$
„	$\frac{4}{10}$	inch	$\frac{1}{1000}''$	„	$\frac{1}{2}$	inch	$\frac{1}{2000}$
Dancer	2	inch	$\frac{1}{200}$	„	$\frac{1}{4}$	inch	nil
„	$\frac{1}{15}$	inch	nil	„	$\frac{1}{8}$	inch	nil

In Beck's recent catalogues, however, it is stated that these maker's objectives can be used without alteration, the two foci

being so nearly coincident, and as far as my limited experience goes I can substantiate the statement.

16. As in ordinary photography many assert that the wet process gives results superior to the dry, but I much doubt this. If there is any difference it is so slight that only an advocate of the wet plate can detect it. Gelatine plates should not be over-exposed, especially in the case of delicate work, or all the finer details will be lost. No precise directions can be given as to the time of exposure necessary for the production of a good negative. The exposure is largely dependent upon the quality of illumination, but even more so upon the nature of the object. By direct sunlight with a not too dense subject, under the strongest magnification and sufficient weakening of the light by a filter a few seconds should suffice. Opaque objects illuminated by reflected light require a much longer exposure than transparent subjects with transmitted light, and the difficulties in focussing increase rapidly with the magnifying power. As a guide to exposure I extract from Davis' Practical Microscopy the following table relating to Mawson and Swan's "15 times" plates :—

Objective.	Subject.	Exposures.	
		With microscope lamp.	With a triplexicon or sciopticon lamp.
4 inch	Wing of blow-fly ...	6 seconds.	3 seconds.
2 inch	Proboscis of do. ...	60 "	10 "
1 inch	Do. do. ...	70 "	20 "
½ inch	Glass crystal	60 "	30 "
¼ inch	Section of deal	7 minutes.	2 minutes.
⅛ inch	Podura scale	10 "	2½ "
$\frac{1}{15}$ inch	Pleurosigma attenuatum ...	15 "	3 "

In recent years instantaneous work has been taken up with a view to photograph moving organisms, and exposures from

$\frac{1}{20}$ to $\frac{1}{200}$ of a second are reported to have been satisfactorily given with immersion lenses. Formerly infusoria, &c., were rendered stationary by killing them with an electric shock. A flash light recommended for instantaneous work is produced by the ignition of the following mixture, which gives a flash lasting from $\frac{1}{50}$ to $\frac{1}{30}$ of second:—

Magnesium	30	parts (by weight)	in powder.	
Chlorate of potash	60	„	„	„
Sulphite of antimony	10	„	„	„

The combustion of this powder is effected in a metallic tube closed at one end and provided at the other with a glass plate and diaphragm, the aperture of which corresponds accurately with the diameter of the illuminating lens. Within the tube and on a level with its central point is a metal plate upon which the powder with a piece of touch paper is placed. The latter is ignited through a slit in the tube closable by a shutter. The tube is further provided with a very long chimney.

17. With long exposures it is very essential that freedom from vibration should be secured and this is a matter of considerable difficulty. Dr. Woodward used to isolate his apparatus from the floor of the room by placing it on solid concrete pillars built up independently from the ground, but even this was not always satisfactory. However long exposures can be made, and photographs of phosphorescent bacilli have been taken by their own emitted light with an exposure of 36 hours and more.

18. As regards development, the same rules as apply to ordinary photography hold with photo-micrography, but in fine work it is not advisable to push development too far lest the more delicate details should be lost. There is no object in giving receipes for developers, as it will be best to adopt the formula which the operator is in the habit of using for ordinary work.

19. Having briefly touched on the most important points connected with photo-micrography I will conclude this paper by pointing out that this branch of our hobby possesses the great advantage that it can be practised in the evenings when it will be found an excellent alternative to the long-arm-chair, and that being independent in a great measure of conditions of weather it can be indulged in when other photographic work would be impossible.

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